How reliable is morphological species delimitation in kelp? A study of two closely related South African *Ecklonia* species.

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ABSTRACT

Ecklonia maxima and *Ecklonia radiata* are both kelp bed forming macroalgae along the South African coast, and the latter is also found in considerable abundance in Australia and New Zealand. Genetically they exist as two distinct species and can usually be differentiated morphologically, especially when occurring as geographically separate entities. However, they do appear to intergrade when growing together, where plants of apparent intermediate and indeterminate morphology have been observed. This study tested the reliability of morphology in separating these two *Ecklonia* species across their intraspecific range of morphological variation, from locations where the species co-occur, as well as where they appear in isolation in South Africa (both species) and Australia (*E. radiata* only). No individual characters reliably separated between species, yet overall size distinctions as well as the morphometric separation of hollow and solid-stiped sporophytes provide good evidence for morphological differentiation of *E. radiata* and *E. maxima*. While *E. radiata* clusters morphometrically, a distinction between Australian and South Africa specimens is observed. In localities where the two species are reported to co-exist morphological distinction is less clear, particularly in deep water at Buffels Bay. The blade morphology of these deep water sporophytes is distinct from both *E. radiata* and *E. maxima* across all locations, while overall size distinctions contribute most prominently to the morphological separation of *E. radiata* and *E. maxima* at De Hoop. Environmental data in combination with more detailed genetic analyses, especially those aimed at hybrid identification, are necessary to resolve the nature of these subtidal plants as well as to investigate the relationship between genetic differentiation and overlapping morphology in plants at De Hoop.

INTRODUCTION

South African has an extensive coastline of around 3000km (Griffiths et al. 2010), home to a high diversity of marine fauna and flora. Spanning three distinct biogeographical provinces, sensu Stephenson (1948), the cool-temperate west coast, warm-temperate south coast and subtropical east coast each play host to a distinct, although overlapping range of organisms. Seaweeds are often dominant in these rocky intertidal and shallow subtidal systems (Anderson et al. 2009), playing critical roles in ecosystem function as well as providing a multitude of services utilised by humans.

Kelp Ecology

Kelp is the common name given to the group of large, brown seaweeds of the order Laminariales (Phaeophyceae) that dominate rocky coastlines throughout temperate and Arctic waters (Steneck et al. 2002, Smale and Wernberg 2013). The larger kelps can form dense beds or forests over extensive areas (Bolton 2010), creating complex benthic ecosystems that are among the most productive on earth (Smale and Wernberg 2013). Kelp forest development, persistence and distribution is strongly influenced by both the physical and biotic environment, as they are restricted by light conditions, temperature, nutrient levels, the frequency and intensity of storm events as well as being strongly affected by herbivore outbreaks (Smale and Wernberg 2013). Multiple lines of evidence indicate that seaweed geographical distribution is overwhelmingly controlled by seawater temperature (Breeman 1988, Bolton and Anderson 1987, Lüning 1990, Anderson et al. 2007). Accordingly kelps are generally described as temperate and arctic organisms, however, they can survive in the geographical tropics under conditions that satisfy temperature and light requirements, in deeper water where there is enough light, or associated with upwelling regimes of cooler waters (Graham et al. 2007, Bolton 2010).

Dominant kelp genera and species vary across the world's oceans and bioregions, yet globally kelp forests share similarities in structure and function (Smale and Wernberg 2013). Kelps are true ecosystem engineers altering the physical environment with regards to water flow and the hydrodynamic environment (Duggins et al. 1990, Steneck et al. 2002), light (Steneck et al. 2002), and

sedimentation (Eckman et al. 1989, Duggins et al. 1990, Wernberg and Thomsen 2005), while profoundly affecting a myriad of associated biota including marine mammals, fishes, crabs, molluscs as well as other algae (Mann 1973). The physical structure of the plants creates a three-dimensional habitat as well as a nursery ground (Anderson et al. 1997) for both pelagic and benthic organisms. Modification of immediate, nearshore and offshore environments and ecologies through enhanced secondary productivity and allochthonous food input (Duggins et al. 1989, Mann 2000, Steneck et al. 2002, Krumhansl and Scheibling 2012) creates ecosystems capable of supporting high levels of biodiversity and biomass (Dayton 1985, Steneck et al. 2002). Many species are important in industry for human food, production of alginates as well as in medicine and feed in abalone aquaculture (Bolton 2010), while numerous commercially valuable marine fauna are associated with kelp beds (Smale and Wernberg 2013).

Taxonomy

With 112 species distributed among 33 genera (Bolton 2010), the taxonomic diversity of the Laminariales may be described as relatively limited (Steneck et al. 2002), where a greater number of genera are monotypic (Bolton 2010), with only a few that may be termed speciose. No commonly accepted species concept exists for kelps, where the majority have been described based on limited morphological and anatomical features of the sporophyte (Bolton 2010). However, the physiological and ecological significance of these few characters varies (Hurd 2000, Wernberg et al. 2003), and macroalgae are known to exhibit a great deal of morphological variation (Wernberg and Vanderklift 2010), at an interspecific (Roberson and Coyer 2004) and to a lesser degree integeneric (Steneck et al. 2002) level.

Morphological Plasticity

The evolutionary consequences of morphological variation remains largely unstudied (Roberson and Coyer 2004), with regards to genetic differentiation of dissimilar morphotypes of a single or nascent species along a divergent environmental gradient. Roberson and Coyer (2004) conducted morphometric and genetic analyses in combination with laboratory and field transplants of the kelp

Eisenia arborea Areschoug, which has been proposed as *Ecklonia arborea* (Areschoug) Rothman, Mattio & Bolton *comb. nov.* by Rothman et al.(in press). They studied two distinct morphs from adjacent low and high water flow areas to determine the underlying nature of morphological variation. They found a correlation between water flow and morphology, significant genetic differentiation between the two morphs (using M13 DNA fingerprinting), as well as demonstrating that transplanted individuals retained the morphology of the original collection site. This indicates that the blade morphology of *E. arborea* is genetically fixed and not a plastic response to the local environment. Similarly, Miller (2000) found significant genetic differentiation in the kelp *Pelagophycus porra* (Léman) Setchell between genetically dissimilar morphotypes along an environmental gradient.

However, Fowler-Walker et al. (2006), in reciprocal transplant experiments of *Ecklonia radiata* (C. Agardh) J. Agardh between sheltered and exposed environments found rapid changes in morphology in exposure related traits, particularly in individuals transplanted to high flow environments. This suggested that morphological plasticity rather than genetic differentiation is the cause of different patterns in morphology between environments in this species in South Australia. Intraspecific phenotypic variation of individual characters may thus be under genetic or environmental control (Roberson and Coyer 2004), if not a combination of these factors (Wernberg and Thomsen 2005, Wernberg and Vanderklift 2010), however this is not well understood.

Kelp species are highly plastic (Bolton 2010, Martin and Zuccarello 2012), and modify their environment as a function of their morphology (Velimirov and Griffiths 1979, Kennelly 1989) providing a structurally variable habitat in space and time with effects on associated biodiversity. Morphological variability is believed to increase kelp fitness and survival (Wernberg and Vanderklift 2010, Blanchette et al. 2002) by providing a mechanism for niche expansion within a species (Gerard and Mann 1979), where the resultant phenotype represents a trade-off between photosynthetic ability, nutrient uptake and structural resilience (Blanchette et al. 2002). Morphological variation is clearly an important feature of kelps, yet both the underlying mechanisms and effects remain unclear.

Study genus

The genus *Ecklonia* Hornemann belongs to the family Lessoniaceae and has a widespread distribution across warm- temperate oceans of both hemispheres (Bolton and Anderson 1994, Steneck et al. 2002, Rothman et al. in press). Amongst the most warm tolerant of all kelps (Bolton and Levitt 1985, Bolton and Anderson 1987, Bolton 2010), it often inhabits regions where summer monthly mean temperatures are over 20°C, but does not occur in true cold-temperate conditions where minimum monthly mean temperatures fall below 10°C (Bolton and Anderson 1994). Ecklonia plants are commonly ecologically dominant (Bolton and Anderson 1994), forming large subtidal kelp forests in the Southern Hemisphere and the northwest Pacific (Martin and Zuccarello 2012).

Southern Hemisphere species of *Ecklonia* are morphologically fairly similar (Bolton 2010), with a single spreading holdfast, a simple stipe that may be short to very long, either solid or inflated in parts (Bolton and Anderson 1994), and a primary blade with secondary blades on each side. Blade morphology is somewhat variable, being long, narrow and strap-like to wide and more amorphous, with a surface that may be smooth, rugose, corrugated or spinulose, and margins that may be more or less spinulose with teeth or spines (Bolton and Anderson 1994).

Study Species

The species of interest in this study are *Ecklonia maxima* (Osbeck) Papenfuss and *Ecklonia radiata*, both of which are kelp bed forming macroalgae along the South African coast (Bolton et al. 2012, Bolton and Anderson 1987). *Ecklonia maxima* dominates on the southern west coast of South Africa (Anderson et al. 2009, Bolton et al. 2012), forming large kelp forests in the Benguela upwelling marine ecosystem (Bolton and Anderson 1994, Bolton and Anderson 1997, Bolton 2010, Bolton et al. 2012). *Ecklonia radiata* has a wider distribution both within South Africa, and globally, found along the south and east coasts of South Africa, as well as dominating many temperate reefs in Australia (Steinberg and Kendrick 1999), and New Zealand (Wing et al. 2007).While both are habitat forming kelps, *E. radiata* is much less abundant (Rothman et al. in press), and the beds are not fully equivalent

in form and function to the enormous *E. maxima* forests found along the southern west coast of South Africa.

Traditional morphological descriptions of *E maxima* indicate a long stipe (up to 10m), hollow in mature plants and terminated with a gas filled bulb or float holding the distal end at or near the surface of the water (Stegenga et al. 1997, Bolton et al. 2012). The blades are generally smooth in mature plants, with margins that may also be smooth or corrugated. *Ecklonia maxima* does not grow where annual mean temperatures exceed 16°C and maximum monthly mean temperatures exceed 20°C. *Ecklonia radiata* has a solid stipe, generally much shorter than that of *E. maxima* at up to 1 m (Stegenga et al. 1997), although it can reach a maximum of 2 m (Wernberg et al. 2003, Mabin et al. 2013). The blades may be more or less spinulose, or completely smooth, although the ecological significance of the spines remains unknown (Wernberg and Vanderklift 2010). This species does not occur in South Africa where annual mean temperatures exceed 20°C and maximum monthly mean temperatures exceed 22°C (Bolton and Anderson 1987, Bolton 1986).

The name *E. radiata* is given to a wide variety of morphologies (Bolton and Anderson 1994), with a confused taxonomic history, primarily due to the high degree of intraspecific morphological variation. Genetic analyses have confirmed their existence as a single species in the Southern Hemisphere (Rothman et al in press), encompassing a broad morphological description of the species including those with and without spinose blades (Bolton and Anderson 1994, Wing et al. 2007, Martin and Zuccarello 2012). The presence of intermixed and intergrading morphological variants within a single locality or on a small spatial scale suggests that a phenotypically plastic response to environmental conditions is primarily responsible for the wide variation in morphology rather than genetically fixed differences (Fowler-Walker et al. 2006, Wernberg and Vanderklift 2010).

These two species show different growth and reproduction optima at different temperatures in laboratory culture (Bolton & Anderson 1987, 1994), also displaying historically different ranges in South Africa associated with different geographical provinces. *Ecklonia maxima* was found only to the west of Cape Agulhas, while *E. radiata* was found growing from about 60km east of Cape

Agulhas in shallow water as far east as Port Edward on the East Coast (Bolton and Anderson 1987). *Ecklonia radiata* grows in deeper water further north, as far as the South Africa/Mozambique border (DeClerck et al. 2005)

Sometime between 2006 and 2008, *E. maxima* was discovered growing in some abundance in the shallow subtidal and lining the walls of shallow gullies at the De Hoop Nature Reserve (Bolton et al. 2012). This was an eastward range extension, where it now grows alongside *E. radiata*. Additionally, non-spinous plants thought to be *E. radiata* co-exist with *E. maxima* off Buffels Bay, on the east coast of the Cape Peninsula near Cape Town (Stegenga et al. 1997). The morphology of these subtidal plants appears as intermediate between the two species, it being difficult to distinguish between an *E. radiata* plant and an *E. maxima* plant before the bulb is developed (Bolton and Anderson 1994).

Morphologically the two species can usually be differentiated from each other, especially when occurring as geographically separate entities. However, they do appear to intergrade when growing together, where plants of apparent intermediate and indeterminate morphology have been observed. Using four genetic markers (ITS, rbcL, atp8, trnWI) across nuclear, mitochondrial and chloroplastic DNA sequences, Rothman et al. (in press) confirmed the existence of *E. maxima* and *E. radiata* as distinct species in South Africa. Molecular methods therefore appear to reliably separate these two species, although an exception in a deep water Buffels Bay specimen was seen, with mixed results in molecular analyses.

Speculations as to the close relationship between *E. maxima* and *E. radiata* were strengthened in a study by Bolton and Anderson (1987), who crossed gametophytes of both species under laboratory conditions, forming hybrid sporophytes with intermediate temperature tolerances between their parent species. While long term sporophyte persistence was not monitored, there is the possibility for hybrids of the two species to exist in nature. There is a hypothesis that *E. maxima* evolved from *E. radiata* type in fairly recent geological time (Bolton and Anderson 1994, Matson and Edwards 2006), supported by the adaptation of *E. maxima* to the low temperature and high nutrient conditions of the Benguela current on the west coast of Southern Africa (Bolton and Levitt 1987) as it has been

suggested that this upwelling is of relatively recent origin, having begun within the last 3 million years (Marlow et al. 2000).

Research Aims

The main question addressed in this study is whether it is possible to separate these two *Ecklonia* species across their intraspecific range of morphological variation, testing traditional species descriptions. In South Africa individuals from localities where *E. maxima* and *E. radiata* co-exist will be examined, as well as where they occur in isolation in South Africa, while in Australia *E. radiata* from two sites exhibiting a range of different morphologies will be analysed. In testing for morphological differences between the two species, the morphological characteristics that best distinguish them will be analysed, as well as testing for plants of intermediate morphology and where they occur.

E. maxima and *E. radiata* are genetically different species (Rothman et al. in press). Therefore it is expected that they will separate morphometrically. Bolton and Anderson (1987) demonstrated that the two species can hybridise in the laboratory, and given their recent range overlap on the south coast, and possible historical overlap at Buffels Bay, we hypothesise that some plants will display morphology that is intermediate between the two. Finally molecular data indicate that South African and Australian *E. radiata* are the same species, therefore, it is hypothesized that they will cluster together morphometrically.

The spread of *E. maxima* along the coast of South Africa and recent overlap of these species at De Hoop, as well as the potential co-existence off False Bay may have implications for the maintenance of these species as individual entities. How kelps modify their environment is a function of their morphology and thus any variation or change will affect not only local but neighbouring ecosystems. Additionally, observable impacts due to climate change have already been seen on kelp forests (Steneck et al. 2002, Smale and Wernberg 2013), while human induced stressors including fishing and resource exploitation, coastal land use and pollution interact with these effects degrading the services that these highly productive systems provide. An understanding of the nature and effects of

morphological variation forms an important component in understanding function of these ecosystems, and thus their resilience to both anthropogenically induced environmental change and human impacts.

METHODS AND MATERIALS

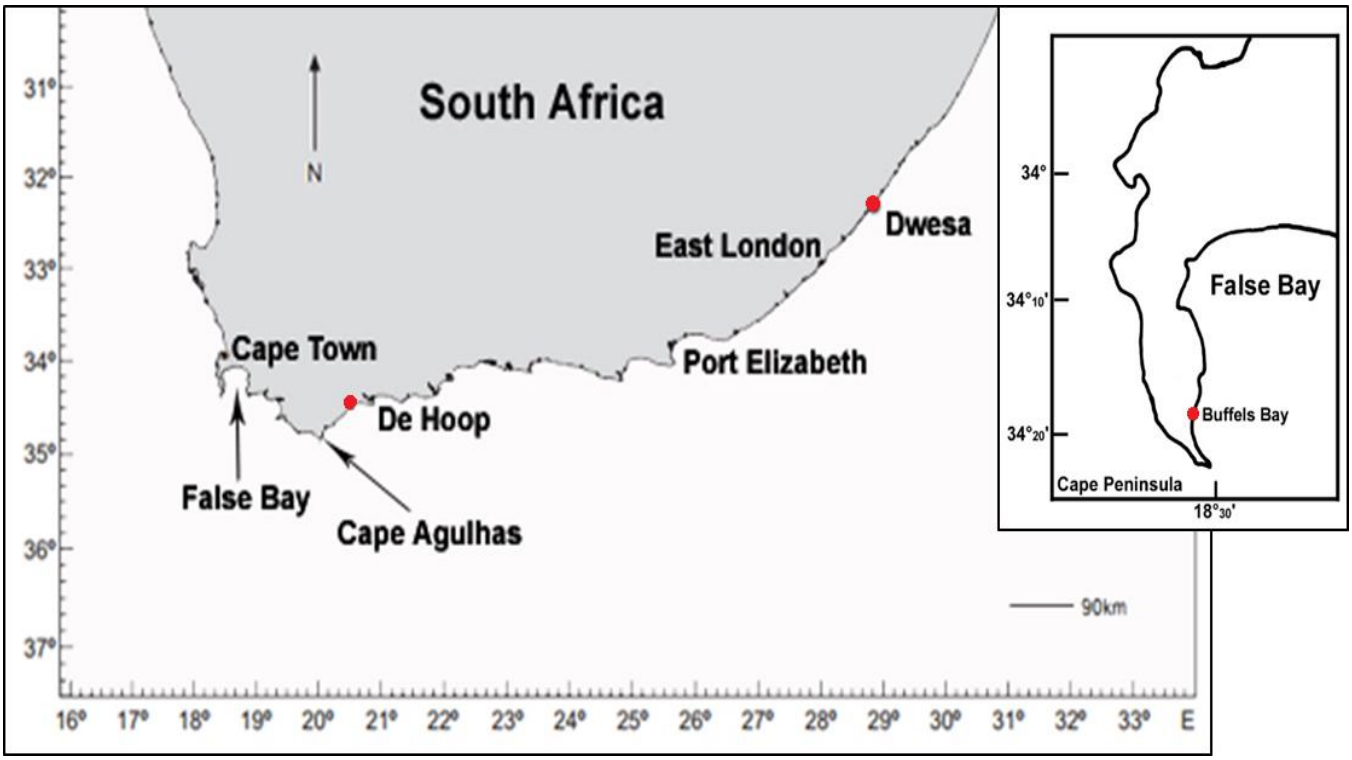

Figure 1: Map of South Africa showing the sampling locations (detailed in red) mentioned in the text. Inset shows location of Buffels Bay within False Bay. SA map adapted from Robinson et al. 2005.

Study Sites

Sites were selected based on the occurrence of the two species as either geographically separate entities or where they have been described to occur together at two sites within South Africa. Data on South African *Ecklonia* plants and sites of extreme variation in *E. radiata* from Western Australia were also used, collected by Dr Thomas Wernberg of the University of Western Australia. A summary of sites and species at each site may be seen in Table 1.

The first site, Koppie Alleen, is situated within the De Hoop Nature Reserve (34°26′S 20°32′E) approximately 60km east of Cape Agulhas on the south coast of South Africa. This 46km area of

coastline was declared a Marine Protected Area in 1986. The shore consists of sandy beach and intertidal aeolianite rock ledges exposed to high wave action (Bennett and Attwood 1993, Bolton et al. 2012). As a result, erosion of these ledges has produced a complex substratum composed of a sandy sea bed interspersed with rock patches of varying sizes (Bennett and Attwood 1991). Accordingly the sand is highly mobile and covers and exposes rock on varying temporal and spatial scales with important consequences on invertebrate and algal communities.

Table 1: Regional, species and depth information for each sampling location. Key indicates names given to sampling locations in Figures 4-7 and Table 4.

Key	Location	Species	Depth
Buffels	Buffels Bay, South Africa	*E. maxima*	ca. 2 m
De Hoop	De Hoop Nature Reserve, South Africa	*E. maxima*	ca. 2 m
		E. radiata	ca. 2 m
Dwesa	Dwesa Nature Resrve, South Africa	*E. radiata*	ca. 2 m
Aus_Mew	Mewstone Rock, Western Australia	*E. radiata*	6 - 12 m
Aus_Cas	Casuarine Shoal, Western Australia	*E. radiata*	6 - 12 m
DeepBuffels	Buffels Bay, South Africa	*E. maxima*	8 m
		E. radiata?	16 m

The second site, Buffels Bay (34°19′S, 18°27′E), is located on the shore of False Bay within Cape Point, part of the Table Mountain National Park. This is part of an overlap region between the south and west coast intertidal fauna and flora of South Africa, extending from the Cape Peninsula to Cape Agulhas (Stephenson 1939, Anderson et al. 2009). Wave action in the Cape of Good Hope is generally high, and Buffels Bay is considered moderately exposed (McQuaid and Branch 1985) in a South African context. The substrate is composed of Table Mountain Sandstone, which weathers slowly in a horizontal manner creating broad beaches with a gentle slope (McQuaid and Branch 1984). The east coast of the Cape Peninsula generally experiences warmer temperatures than the west coast, and Buffels Bay falls within the warmer water sites of False Bay where mean monthly sea temperatures range from 15.6 to 17.4°C in summer and from 14.2 to 15°C in winter (unpublished data supplied by the Department of Agriculture, Forestry and Fisheries).

The third site, Dwesa (32°18′S, 28°50′E), is located within the Dwesa Nature Reserve and lies on the east coast of South Africa. This Marine Protected Area was declared in 1978 and covers approximately 23km, the majority of which is rocky shore (Branch and Odendaal 2003). The rough coastline is composed of shale rock platforms and steep cliffs and is exposed to continuous moderate to heavy wave action (Dye 1988, Dye and White 1991, Dye 1993). Sea temperatures range from 13 to 24°C, with a mean of 18°C (Dye 1988).

Kelp collection

Sampling of *E. maxima* and *E. radiata* was carried out during the months of April 2014 in De Hoop during low spring tide, *E. radiata* was collected from Dwesa in May 2014, and *E.maxima* was collected during July 2014 in Buffels Bay. Kelps were collected haphazardly from all locations, to include the full range of *Ecklonia* morphological variation at each site. To avoid confounding effects of ontogenetic variation only mature sporophytes were collected (life stage 3, Kirkman 1981), where thalli have fully differentiated branching laterals, and morphology does not change ontogenetically, but is influenced by seasonal growth and erosion of the kelp thallus (Wernberg and Vanderklift 2010).

Because De Hoop is a shallow gradient beach, with little depth difference between habitat types, we limited our sampling to the intertidal (including rock pools) and less than 2 m depth. Sampling between habitat types, including intertidal rock pools, shallow subtidal zone and edge/cliff hanging plants was necessary to encompass the range of morphological variation, due to the absence of a depth gradient. Contrastingly, Buffels Bay displays a larger depth gradient but little difference between habitat types along the shore. Only *E.maxima* was harvested from Buffels Bay at ca. 2 m depth during the month of July.

Both *Ecklonia* species were collected from Buffels Bay during October 2012, including what was termed *E .maxima* at a depth of 8 m, as well as deep water (16 m) sporophytes with relatively long stipes and rugose blades, which following Stegenga et al. (1997) were regarded as *E. radiata*. Additional specimens of *E. radiata* were collected in November 2003 off the coast of Fremantle on the west coast of Western Australia at Mewstone Rock (32°05′S, 115°39′E) and Casuarine Shoal

(32°08′S, 115°36′E), both structurally complex, steep reefs, with a depth range of 6 to 12 m (Wernberg and Vanderklift 2010).

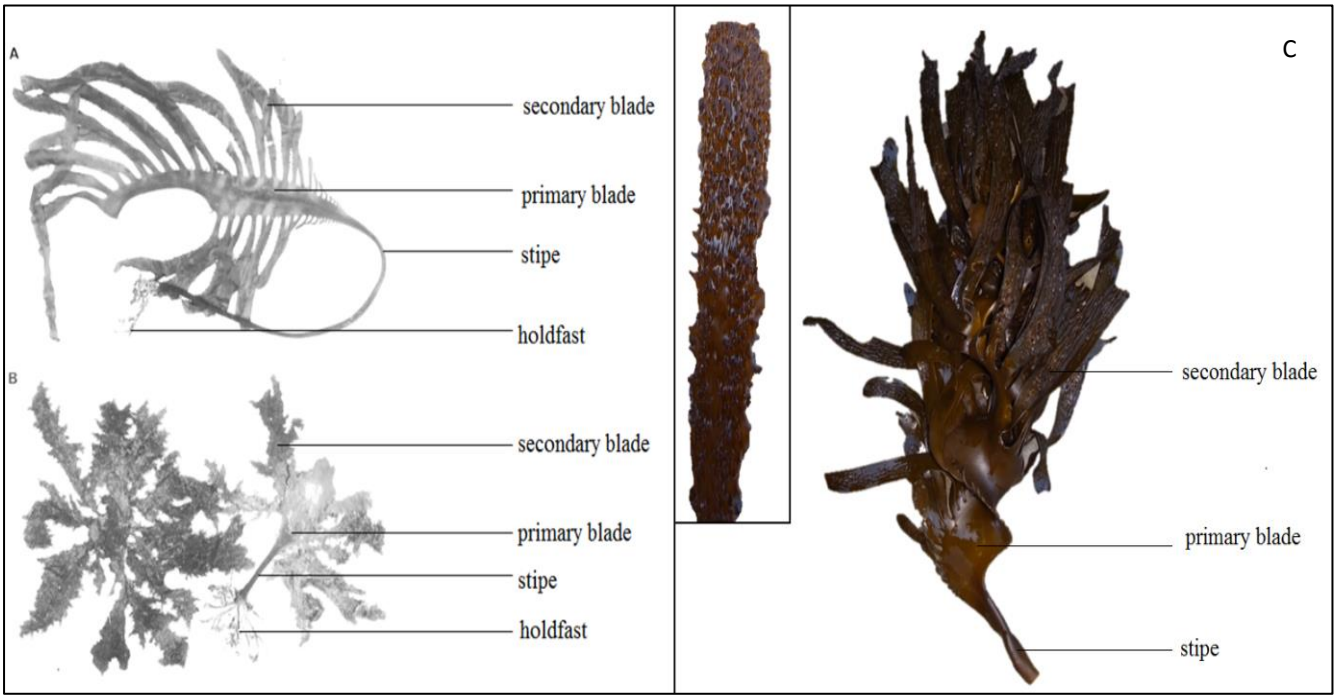

Figure 2: General morphology of *E. radiata* indicating parts of the plant, showing variability in form. A: smooth bladed with long stipe, long primary and multiple secondary blades, B: Spinose morphology with short primary blade, fewer secondaries and short stipe. *E. radiata* specimen (C) demonstrates a twist in the primary blade, as well as rugosity in secondary blades, and inset shows spinose secondary blade. Adapted from Womersley (1967).

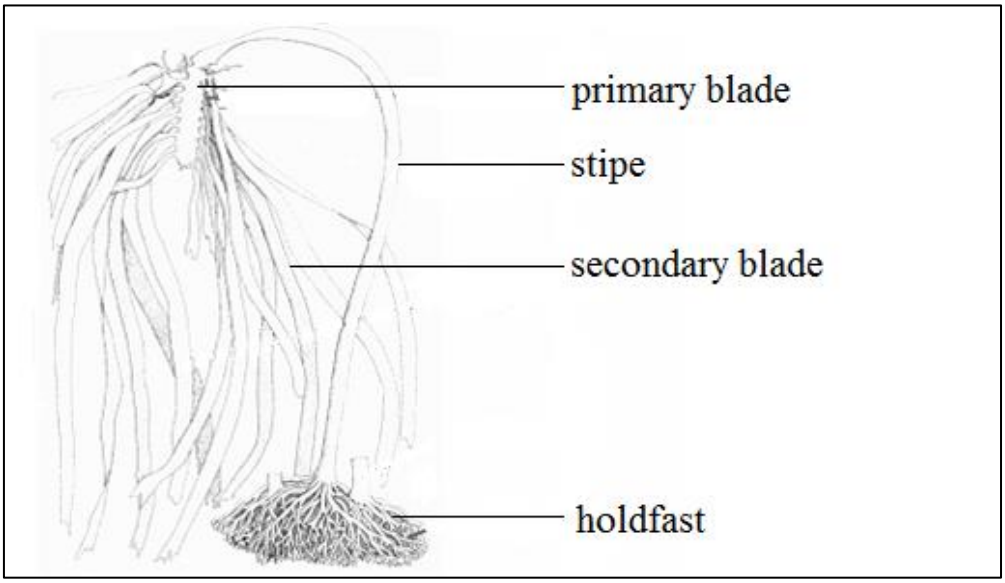

Figure 3: General morphology of E. maxima indicating parts of the plant. Adapted from Stegenga et al. 1997.

Measurements and analysis

Table 2: List of morphological characters, measurement units and procedures for all plants collected by The University of Cape Town at De Hoop, Dwesa and Buffels (ca. 2m) in 2014.

Morphological Character		Units	Methods/Procedure
Frond Length		cm	Longest frond selected
Frond Weight		g	Total weight of frond
Stipe Length		cm	Immediately above holdfast to where stipe widens and flattens into frond
Stipe Weight		g	Total weight of stipe
Stipe diameter	At base	mm	Immediately above holdfast
	At widest point		Where widest
Primary Blade Length		mm	From where stipe widens and flattens into frond to distal end of primary blade
Primary Blade Width		mm	At maximum
Primary Blade Thickness		mm	At maximum
Primary Blade Twists		count	No. of full rotations of the central blade
Number of Secondary Blades		count	Counted on one side of Primary Blade
Secondary Blade Width		mm	Width of blade selected for length at widest point
Secondary Blade Thickness		mm	Thickness of selected blade
Rugosity		ripples. cm^{-1}	Taken from where secondary blade width was measured
Spinuosity		spines. cm^{-2}	No. of pointed protrusions (spines) on the blade surface
Fertility		Presence/absence	Presence of visible sori
Crenulations/Ridges		number. 10cm	Presence on edge of blade
Epiphytes		Presence/absence Species/type Percentage coverage	Species identified if possible. Alternatively algae type (crust/foliose identified. Percentage cover estimated
Length of hollow		cm	Length of stipe that is hollow
Inner diameter		mm	Max width of inner hollow part
Outer diameter		mm	Max width of outer hollow part at point of inner diameter measurement

Thirty *E. maxima* sporophytes from Buffels Bay, ten *E. radiata* sporophytes from Dwesa and forty two sporophytes from De Hoop including both *E. maxima* and *E. radiata* were collected. Each of the collected plants were numbered and photographed before a young, clean secondary blade, from each

plant was collected and dried in silica gel for later DNA studies (Chase and Hills 1991). In addition one mature frond was also collected for pressing later the same day to serve as a voucher specimen. A number of morphological characters on each kelp thallus were then measured, of which a summary of the methods and morphological characters can be seen in Table 2.

The methods and characters are used and adapted from previous studies (Wernberg et al. 2003, Wernberg 2005, Wernberg and Thomsen 2005, Wernberg and Vanderklift 2010), and can be seen in Table 3. Measurement procedures undertaken at De Hoop, Dwesa and Buffels (shallow) were more detailed than previous *E. radiata* studies, taking quantitative measurements of stipe hollowness, as well as more detailed stipe diameter measurements.

Table 3: Condensed list of morphological characters of *Ecklonia* and measurement units used in analyses of data. Thallus Length is calculated as Frond Length + Stipe Length (see Table 2) and Thallus Weight is calculated as Stipe Weight + Frond Weight (see Table 2). Stipe Diameter refers to diameter at base (see Table 2).

Morphological Character	Units
Thallus Length	cm
Thallus Weight	g
Stipe Length	cm
Stipe Diameter	mm
Primary Blade Length	mm
Primary Blade Width	mm
Primary Blade Thickness	mm
Number of Primary Blade Twists	count
Number of Secondary Blades	count
Secondary Blade Length	cm
Secondary Blade Width	mm
Secondary Blade Thickness	mm
Rugosity	ripples. cm^{-1}
Spinousity	spines. cm^{-2}

Statistical analyses of morphological variables were performed using PRIMER 6 (Plymouth Routines in Multivariate Ecological Research) and the statistical computing and graphics software R (2013). Mentioned routines performed using PRIMER are described in Clarke and Gorley (2006).

All morphological variables were normalised to convert measurements to a comparable scale, after which differences among kelp plants were calculated as Euclidean distances. The multivariate patterns were illustrated by a non-metric multidimensional scaling (nMDS) and principal components analysis (PCA). Clusters indicating resemblance levels were drawn on nMDS plots to aid in group identification. An nMDS plot indicating resemblance levels was also created excluding deep Buffels plants. SIMPER analyses were used to test the relative contribution of each morphological character to differences between sampled locations. One way Analysis of Similarity (ANOSIM) was performed to determine the effect of hollowness on multivariate patterns. A second ANOSIM tested the effects of location.

Regression analyses using R were used to related stipe length to the proportion of hollow stipe. R was also used to construct boxplots of each morphological character.

RESULTS

The morphology of *Ecklonia* plants showed considerable variation within and among locations, with regards to most of the traits measured (Fig. 4, Fig. 5). SIMPER analyses (Table 4) revealed that only a few morphological characteristics contributed greatly to overall morphological differentiation between the groups, (1 - 4 characters accounting for over 50% of observed variation between locations). These characters varied greatly between groups, but none standing out as particularly important across all locations (Table 4).

Overall size characters, thallus length and weight, secondary blade length, as well as stipe length were greatest and most variable at Buffels, followed by Deep Buffels and De Hoop, the latter also displaying a fair amount of variability (Fig. 4). Values for these characters were lower at Dwesa, Casuarine Shoal and Mewstone Rock, as well as less variable. Stipe length (Fig. 4) was greatest at

locations where hollow plants were present, and this trait was much less variable at Casuarine Shoal and Mewstone Rock than at other sites. Both stipe length (10.15 - 18.07%) and thallus length (7.78 - 18.42%) consistently separated shallow water Buffels Bay from all other locations (Table 4).

Certain characters contributed more strongly to separation between locations (Table 4). The exception was De Hoop where differentiating characters between locations were highly variable, and no major traits separating this location from others could be identified. Average Square Distance (ASD), a measure of the average dissimilarity between pairs of sites, between De Hoop and other locations were fairly low and consistent between 18.63 and 25.21 within South Africa, and 29.65 and 36.41 between De Hoop and Australian sites. De Hoop sporophytes appeared to have intermediate values for all aforementioned characters (Fig. 4) when compared with those from Buffels Bay (shallow and deep) and Dwesa, Casuarine Shoal and Mewstone Rock.

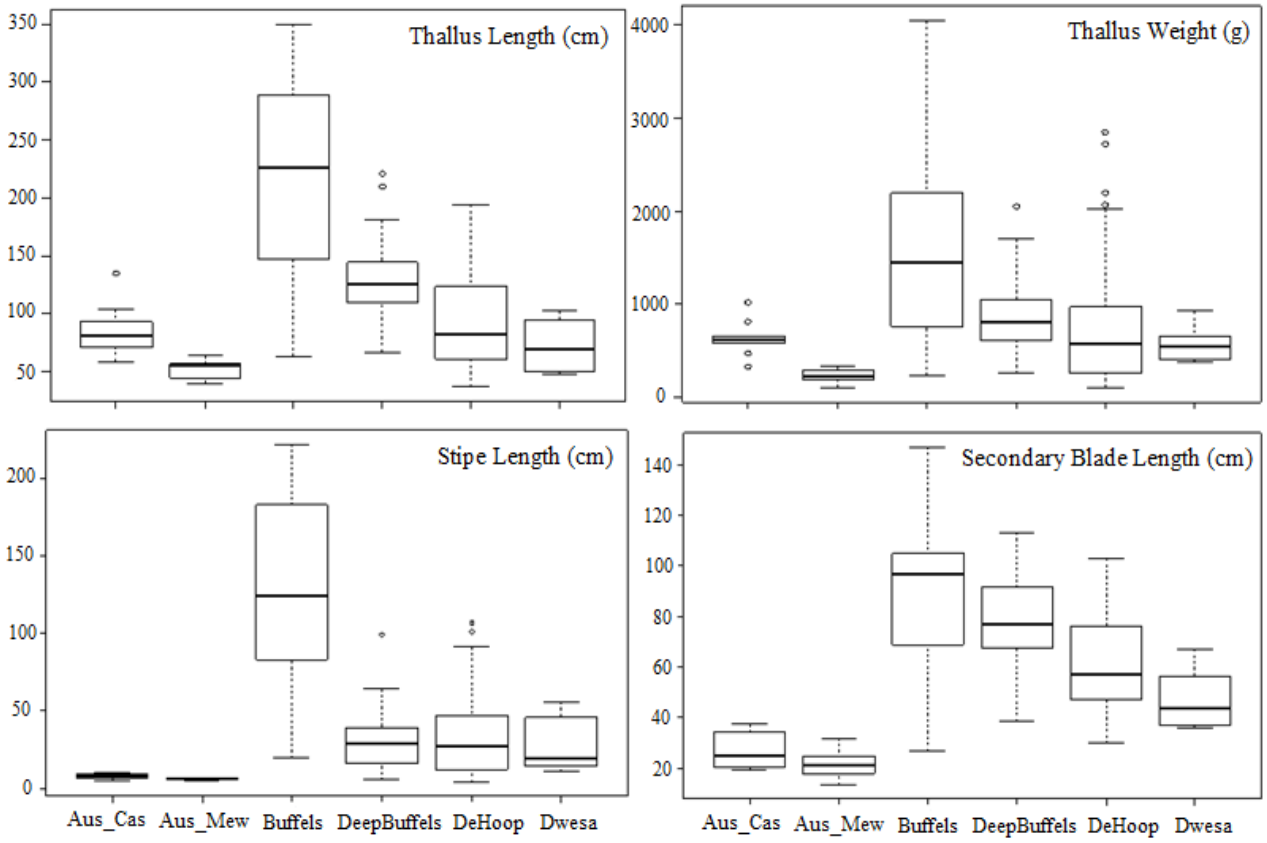

Figure 4: Box plots showing morphological variation in overall size characters of *Ecklonia* plants from each of the six locations sampled. Boxes represent: Aus_Cas (n = 10), Aus_Mew (n = 10), Buffels (n = 30), Deep Buffels (n = 43), De Hoop (n = 42), Dwesa (n = 10). The box represents

minimum and maximum values, the line represents the median, and the whiskers are $25^{th}/75^{th}$ percentiles.

Primary blade length in all locations indicated the same pattern; longer primary blades had a larger number of secondary blades (Fig. 5). The longest primary blades were observed at Casuarine Shoal, an important trait (Table 4), in distinguishing this site from all other locations (17.8 - 51.49%). Mewstone Rock and Dwesa sporophytes also exhibited long primary blades, while sporophytes from the other sites all had similar lengths (Fig. 5). Number of secondary blades (Table 4) was important in separating Dwesa from all other South African locations (21.18% - 35.36%).

Primary blade width did not vary greatly between sites, although they were highly variable at Buffels, and somewhat variable at De Hoop and Deep Buffels. Secondary blade width also did not vary greatly between sites, although an exception at Deep Buffels was seen with more variability around the higher median (Fig. 5), separating this location (Table 4) from others consistently across all sites (8.5 - 19.92%). Secondary blade thickness was similar in median values among locations, although highly variable at deep water Buffels. Primary blade thickness was greatest at shallow water Buffels, lowest at Casuarine Shoal and Mewstone Rock, and somewhat intermediate among the remaining locations.

Mewstone Rock and shallow water Buffels sporophytes had no primary blade twists, while deep water Buffels and De Hoop had very few (Fig. 5). Plants with the most primary blade twists were found at Dwesa, a trait distinguishing this site well (Table 4) from all sampling locations (10.75 - 22.4%). De Hoop and Dwesa are best separated by number of secondary blades and number of primary blade twists (Table 4). Great variability in rugosity was seen in deep water Buffels sporophytes (Fig. 5), separating this site (Table 4) across all locations (7.44 - 15.46%). Casuarine Shoal and Mewstone Rock also exhibited rugose blades, with some associated variability. The most spinose blades were found at Mewstone Rock (Fig. 5), a trait that separates this location well (Table 4) from all locations (20.35 - 44.03%), with some spinose sporophytes at Casuarine Shoal and a small number at De Hoop.

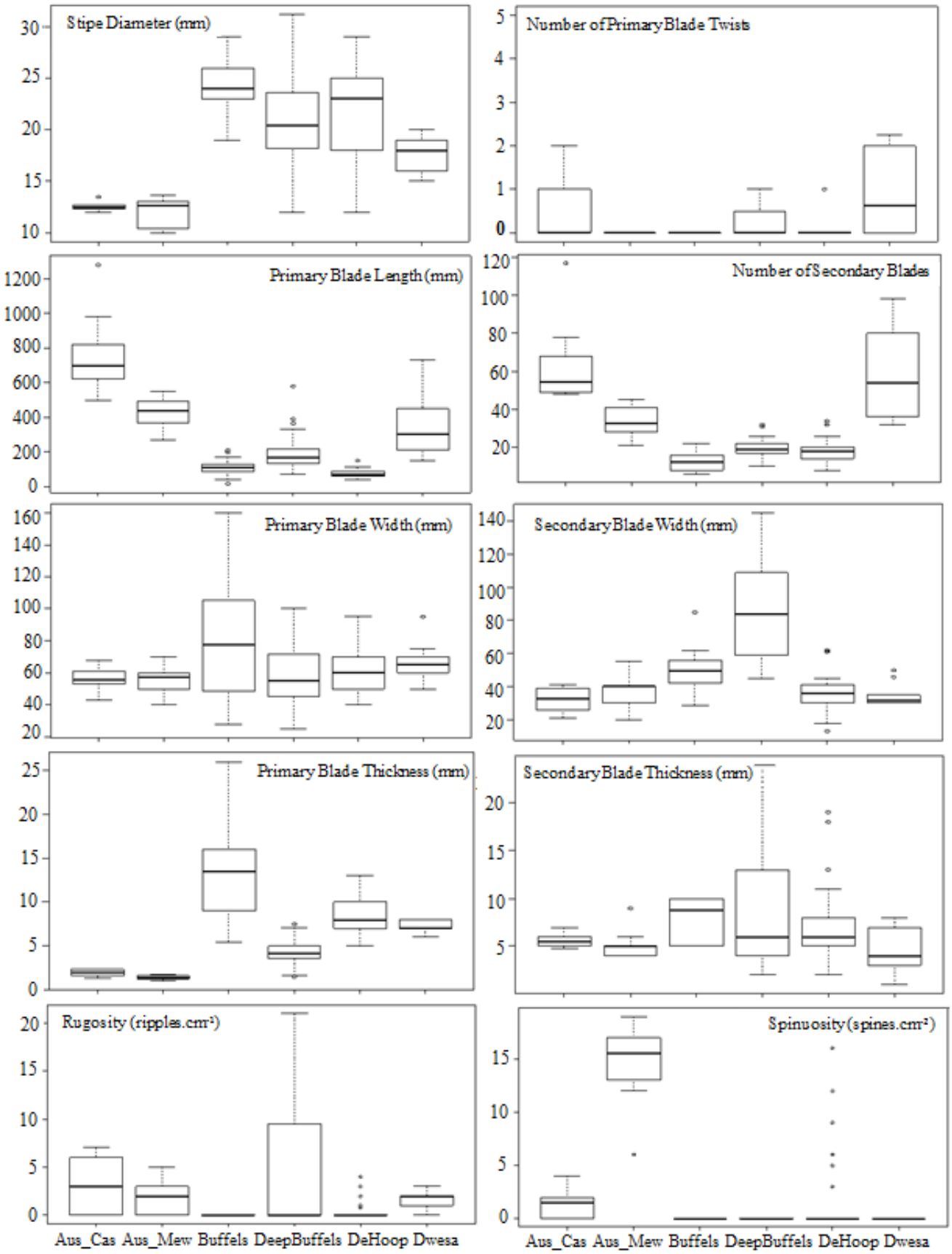

Figure 5: Box plots showing morphological variation of *Ecklonia* plants from each of the six locations sampled. Boxes represent: Aus_Cas (n = 10), Aus_Mew (n = 10), Buffels (n = 30), Deep

Buffels (n = 43), De Hoop (n = 42), Dwesa (n = 10). The box represents minimum and maximum values, the line represents the median, and the whiskers are $25^{th}/75^{th}$ percentiles.

The greatest ASD between sites (Table 4) was observed between shallow water Buffels and Casuarine Shoal and Mewstone Rock at 62.23 and 60.58 respectively. The greatest difference between deep water Buffels plants and other locations was seen with Casuarine Shoal and Mewstone Rock (ASD= 40.66 and 39.70) respectively. Within South African sites, ASD is greatest between Buffels and Dwesa (41.43), followed by deep and shallow water Buffels (33.96) and deep water Buffels and Dwesa (30.5). The ASD (Table 4) between the Australian sites is relatively small (23.12).

In the PCA analysis (Fig. 6, Table 5), the variables that carried the most weight in the first principal component (thallus length, thallus weight, stipe length, and secondary blade length) were related to the overall size of the plants, thus PC1 was henceforth termed the 'size component'. The variables that carried the most weight in the second principal component were primary and secondary blade related characteristics, and thus PC2 was called the 'blade morphology component'.

Figure 6 indicated that kelps from different locations formed somewhat distinct clusters in different regions of this morphospace, although considerable morphological variation was observed within and among locations. Some separation, based on hollowness was also seen, even in localities where the two types (hollow and solid) co-exist. A one way ANOSIM testing for differences between locations ($R = 0.52$, $p = 0.01$) and between hollow and solid ($R = 0.257$, $p = 0.01$) found a significant difference among and between groups.

Plants separated primarily along the first PC axis, those on the right with overall greater thallus length, thallus weight, stipe length, and secondary blade length, including, although to a lesser degree larger stipe diameters and thicker primary blades. These factors caused the spread of some shallow water Buffels Bay sporophytes, illustrated by Group C (Fig. 6), away from the location of 'central' hollow plant morphology. Concurrently it would appear that, in general, hollow plants from both De Hoop and Deep Buffels (included in Group B) and *E. radiata* from Mewstone Rock, Casuarine Shoal, Dwesa and solid plants from De Hoop (Group A) were smaller with regards to these traits, as well as

in overall size. Hollow plants in general appeared larger than solid although this distinction was not as clear in deep water Buffels plants. Both solid and hollow deep water Buffels plants appear larger than those in Group A, yet smaller than other hollow plants. Shallow water Buffels Bay sporophytes overlapped most strongly with the morphology of hollow plants from De Hoop and deep water Buffels. When deep water Buffels plants were removed from nMDS analyses, the groupings of A, B and C were still observed (Fig. 13, Appendix A), indicating a clearer distinction between hollow and solid plants, although Group B contained both hollow and solid types, while Groups A and C were composed of primarily of solid and hollow types respectively.

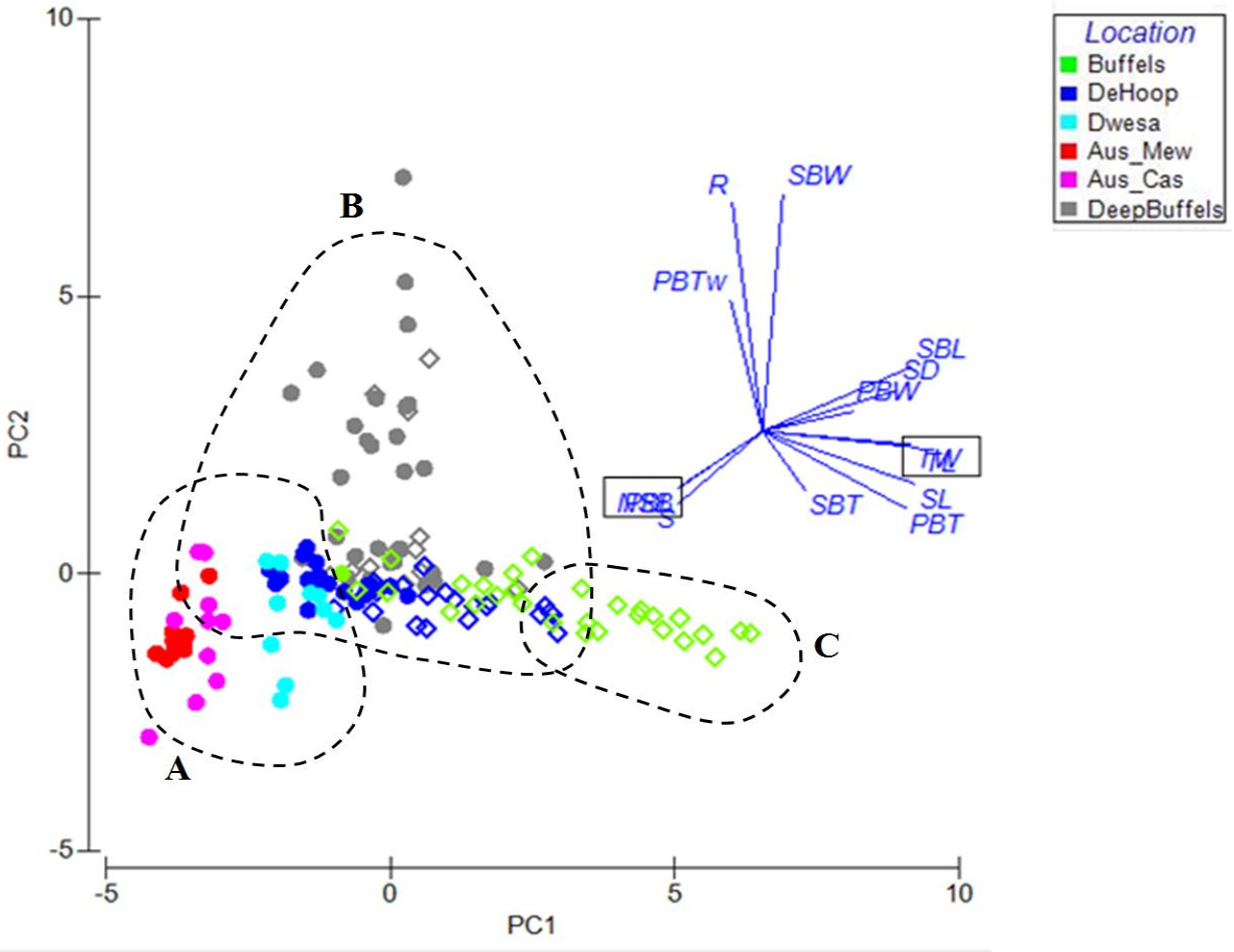

Figure 6: Two dimensional PCA analysis of 14 morphological variables identified in Table 2 for all sampling locations. PC1 accounts for 39.0% of the total variance, and PC2 accounts for 14.8%. Key indicates sampling locations (See Table 3). Each point represents a single plant. The presence (◇) or absence (●) of a hollow stipe is indicated. Dotted lines indicate groups (A, B, C) identified from resemblance levels (distance = 5.6) in MDS (Figure 12, Appendix A).

Morphological characteristics: TL = Thallus Length (cm), TW = Thallus Weight (g), SL = Stipe Length (cm), SD = Stipe Diameter (mm), PBL = Primary Blade Length (mm), PBW = Primary Blade Width (mm), PBT = Primary Blade Thickness (mm), PBTw = Number of Primary Blade Twists, NSB = Number of Secondary Blades, SBL = Secondary Blade Length (cm), SBW = Secondary Blade Width (mm), SBT = Secondary Blade Thickness (mm), R = Rugosity (ripples.cm^{-1}), S = Spinuosity (spines.cm^{-2}). Bottom left box variables: NSB, PBL. Bottom right box variables: TW, TL.

Solid plants displayed more variability in blade morphology compared to hollow plants. In Group A plants, both among and between location variation is seen in blade morphology as well as some separation based on overall size. The latter is less evident in the South African locations. The greatest variation in blade morphology was seen in solid deep water Buffels plants, separated from the all others by highly rugose, wide secondary blades, with many twists on the primary blade, while Group A was composed of plants with many, secondary blades and longer primary blades, with differing degrees of spinuosity.

In locations where the two species have been reported to co-exist (De Hoop and deep water Buffels), morphometric differences between hollow and solid types are evident. Differences in morphological features of the blade between solid and hollow plants were clear in plants from deep water Buffels, while solid plants from De Hoop could be distinguished from hollow on overall size, and to some degree on blade morphology. Solid De Hoop plants form part of Group A (Fig. 6), and overlap most strongly with *E. radiata* from Dwesa. The hollow plants form part of Group B (Fig. 6), and morphological overlap is most evident with hollow plants with from deep water Buffels Bay and *E. maxima* from shallow water at the same location. Overlap between Groups A and B is seen primarily with regards to solid types from De Hoop, and *E. radiata* from Dwesa to a lesser extent.

Solid South African (excluding deep water Buffels) and Australian specimens of *E. radiata* formed visually distinct clusters within Group A. The morphology of the latter is somewhat distinct from South African plants, which are generally both larger in overall size, as well as less spinose, with

fewer, longer secondary blades and thicker, longer primary blades, as well larger in stipe diameter than their Australian conspecifics.

Regression analyses (Fig. 7) reveal a significant, positive relationship between stipe length and the proportion of hollow stipe in shallow water Buffels Bay and De Hoop plants. Plants with similar stipe lengths from each location displayed similar proportions of hollow stipe, especially evident in longer plants (±100cm). Larger plants tend to exhibit greater proportions of hollow stipe, particularly evident in Buffels sporophytes.

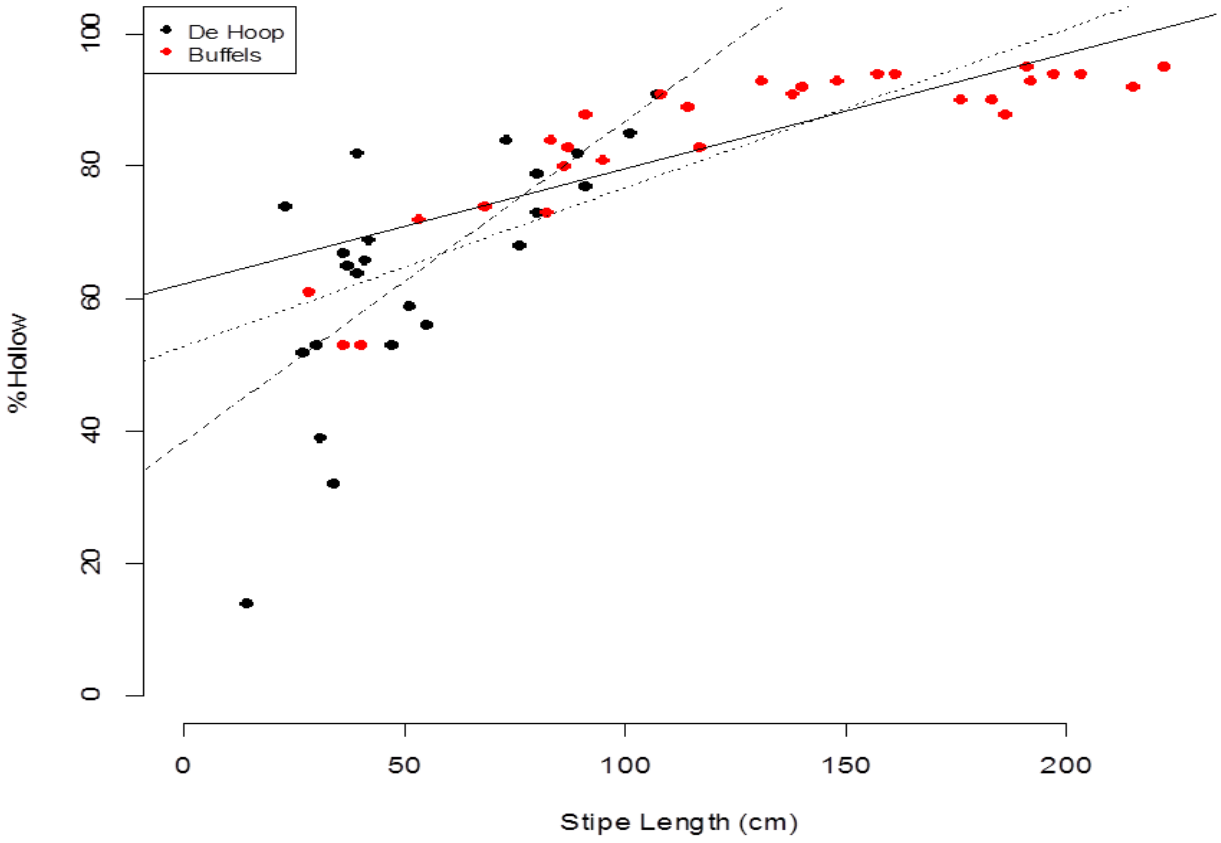

Figure 7: Relationship between stipe length (cm) and percentage of hollow stipe, from two South African sites De Hoop and Buffels, see Table 2 for measurement procedures and Table 3 for key. Regression lines and R^2 values are shown where: De Hoop = dashed and Buffels = solid, both sites = dotted. De Hoop sporophytes are indicated in red, Buffels in black. Only plants with hollow stipes were included in the analyses. Sample sizes: De Hoop (n = 23), Buffels (n = 29).

Rank	Deep Buffels + De Hoop (ASD=21.78)	Deep Buffels + Buffels (ASD = 33.96)	De Hoop + Buffels (ASD = 25.21)	Deep Buffels + Dwesa (ASD = 30.50)	De Hoop + Dwesa (ASD = 21.29)	Buffels + Dwesa (ASD = 41.43)	Deep Buffels + Aus_Mew (ASD = 39.70)	De Hoop + Aus Mew (ASD = 29.65)	Buffels + Aus_Mew (ASD = 60.58)	Dwesa + Aus_Mew (ASD = 27.66)	Deep Buffels + Aus_Cas (ASD = 40.66)	De Hoop + Aus_Cas (ASD = 36.41)	Buffels + Aus_Cas (ASD = 62.23)	Dwesa + Aus_Cas (ASD = 18.99)	Aus_Mew + Aus_Cas (ASD = 23.12)
1	SBW (19.92%)	PBT (14.16%)	TL (18.42%)	NSB (20.63%)	NSB (30.95%)	NSB (20.1%)	S (31.06%)	S (36.08%)	S (20.35%)	S (44.56%)	PBL (23.09%)	PBL (35.05%)	PBL (18.64%)	PBL (30.26%)	S (44.03%)
2	R (15.46%)	SL (13.73%)	SL (18.07%)	PBTw (15.79%)	PBTw (19.64%)	TL (13.37%)	SBL (11.38%)	SD (15.65%)	PBT (12.54%)	PBTw (15.28%)	NSB (17.47%)	NSB (20.4%)	NSB (15.01%)	PBTw (23.02%)	PBL (17.8%)
3	SBT (13.56%)	PBW (11.61%)	TW (15.37%)	SBW (14.36%)	PBL (12.73%)	SL (11.77%)	SBW (10.41%)	PBL (11.03%)	TL (11.26%)	NSB (12.9%)	SBW (11.47%)	SD (11.31%)	PBT (11.29%)	NSB (14.83%)	NSB (17.28%)
4	PBTw (9.53%)	R (10.43%)	PBW (13.7%)	R (11.61%)	SD (7.28%)	PBTw (10.21%)	SD (9.75%)	PBT (8.67%)	SBL (10.98%)	PBT (6.17%)	SBL (9.46%)	PBTw (7.03%)	SL (10.15%)	PBT (7.51%)	PBTw (11.06%)
5	SD (6.77%)	TL (9%)	SBL (9.24%)	SBT (11.07%)	SBT (5.46%)	TW (9.01%)	SBT (7.54%)	SBL (7.77%)	SL (10.69%)	SD (5.21%)	PBTw (9.22%)	PBT (6.14%)	SBL (9.39)	SD (5.91%)	R (3.16%)
6	SBL (5.68%)	TW (8.7%)	PBT (9.21%)	SBL (6.22%)	TW (5.09%)	SBL (8.16%)	R (7.44%)	TW (5.19%)	SD (9.8%)	PBL (4.17%)	SD (8.35%)	SBL (5.11%)	SD (8.54%)	R (5.53%)	TW (1.61%)
7	TW (5.59%)	SBW (8.5%)	SD (4.7%)	PBL (5.63%)	S (4.27%)	PBW (7.19%)	PBTw (5.16%)	NSB (3.26%)	TW (8.58%)	SBL (3.2%)	R (7.46%)	TW (2.85%)	TL (7.78%)	SBL (3.31%)	TL (1.45%)
8	PBW (5.11%)	SBT (7.35%)	SBT (3.72%)	SD (3.95%)	PBW (3.65%)	PBT (6.46%)	PBL (4.55%)	SBT (3.05%)	PBW (5.98%)	PBW (2.51%)	SBT (6.68%)	R (2.6%)	PBW (5.71%)	PBW (3.29%)	PBW (1.18%)
9	PBT (5.02%)	PBTw (6.03%)	S (3.6%)	PBW (3.63%)	SBL (3.62%)	PBL (5.54%)	TL (3.59%)	TL (2.76%)	PBL (4.46%)	R (1.5%)	PBW (1.88%)	S (2.36%)	TW (5.64%)	SBT (1.72%)	SBW (0.76%)
10	S (4.17%)	SBL (4.45%)	SBW (1.95%)	TL (2.98%)	TL (2.79%)	SD (4.63%)	TW (2.72%)	PBW (2.23%)	NSB (2.8%)	SBT (1.27%)	TL (1.61%)	SBT (2.04%)	PBTw (4.11%)	SL (1.19%)	SBT (0.65%)
11	TL (3.76%)	SD (3.86%)	NSB (1.07%)	PBT (1.77%)	SL (1.97%)	SBT (2.45%)	NSB (2.13%)	SL (2.08%)	SBT (1.19%)	TW (1.04%)	TW (1.08%)	PBW (1.61%)	R (1.69%)	S (1.09%)	SBL (0.51%)
12	PBL (2.71%)	PBL (1.3%)	R (0.35%)	TW (1.66%)	PBT (1.33%)	SBW (1.12%)	PBW (2.12%)	R (1.28%)	SBW (0.7%)	SL (0.91%)	PBT (0.94%)	SL (1.6%)	SBW (0.88%)	TL (1.06%)	SD (0.4%)
13	SL (2.02%)	NSB (0.89%)	PBL (0.34%)	SL (0.71%)	SBW (0.82%)	R (0%)	PBT (1.29%)	SBW (0.72%)	R (0.69%)	TL (0.7%)	SL (0.78%)	TL (1.4%)	SBT (0.83%)	TW (0.64%)	PBT (0.09%)
14	NSB (0.7%)	S (0%)	PBTw (0.27%)	S (0%)	R (0.41%)	S (0%)	SL (0.88%)	PBTw (0.23%)	PBTw (0%)	SBW (0.58%)	S (0.51%)	SBW (0.5%)	S (0.33%)	SBW (0.64%)	SL (0.01%)

ASD, Average Square Distance

Table 4: SIMPER analysis showing each morphological character's relative contribution (%) to differences between locations. Morphological characteristics: TL = Thallus Length (cm), TW = Thallus Weight (g), SL = Stipe Length (cm), SD = Stipe Diameter (mm), PBL = Primary Blade Length (mm), PBW = Primary Blade Width (mm), PBT = Primary Blade Thickness (mm), PBTw = Number of Primary Blade Twists, NSB = Number of Secondary Blades, SBL = Secondary Blade Length (cm), SBW = Secondary Blade Width (mm), SBT = Secondary Blade Thickness (mm), R = Rugosity (ripples.cm^{-1}), S = Spinuosity (spines.cm^{-2}).

DISCUSSION

This study included sporophytes of two genetically distinct *Ecklonia* species (Rothman et al. in press), *E. radiata* and *E. maxima*, across their range of intraspecific morphological variation, from locations where the species co-exist, as well as where they appear in isolation in South Africa (both species) and Australia (*E. radiata* only). Morphological variation in kelps has resulted in a great deal of taxonomic confusion (Kain 1979), especially with regards to lower level resolution of morphologically similar or cryptic species. Historically *Ecklonia* species have been diagnosed primarily on external morphology, although the advent of molecular techniques has resulted in increasing clarity of taxonomic relationships within the genus (Lane et al. 2006, Wing et al. 2007, Bolton 2010, Rothman et al. in press).

Traditional Species Description Testing

Characters used to distinguish between *E. maxima* and *E. radiata* are primarily based on stipe morphology, which is described as long and hollow in *E. maxima* and short and solid in *E. radiata* (Bolton and Anderson 1987, Bolton and Anderson 1994, Bolton et al. 2012). Stipe hollowing has been documented in several genera within the Laminariales, likely a phenotypic response to local environmental conditions, perhaps allowing canopy access to better light conditions through increased buoyancy or increased flexibility to reduce effects of wave exposure (Matson and Edwards 2006). While the cause(s) are unclear (Matson and Edwards 2006), difficulties in species delimitation based on stipe related characters including both length and hollowness have been documented in a number of genera due to overlap in variation (Chapman 1973) and environmentally induced variability of these characters (Chapman 1973, Kain 1979, Erting et al. 2004) as well as hybridisation between nascent species (McDevitt and Saunders 2010).

Matson and Edwards (2006) demonstrated that taller *E. arborea* plants were more likely to show stipe hollowing, and a similar relationship in South African hollow plants is seen (Fig. 7). Stipe length separated shallow water Buffels plants (known *E. maxima*) from all other locations, however, this included deep water Buffels and De Hoop where *E. maxima* is also present. While the presence of *E.*

radiata at De Hoop, and possibly deep Buffels may explain separation at these locations, it appears that in isolation stipe length is not a reliable indicator of species. However, overall size (Fig. 4, Fig. 6), including stipe length, appears to be a better predictor of species, except perhaps in deep water Buffels specimens.

A significant separation of hollow and solid plants (Fig. 6) supports traditional species descriptions of *Ecklonia*, although this distinction is less clear in locations where solid and hollow plants co-exist, particularly in deep water at Buffels Bay. Removal of these deep water Buffels plants from the multidimensional analysis (Fig. 13, Appendix A) clarifies this separation. Intraspecific variation in both frequency and extent of hollowness within and between populations of the closely related kelp *E. arborea* (Matson and Edwards 2006, Rothman et al. in press), as well as within some *Laminaria* Lamouroux (now *Saccharina* Stackhouse) populations (Chapman 1973, Kain 1976, Lane et al. 2006, McDevitt and Saunders 2010), could indicate that these solid deep water specimens in Buffels Bay are *E. maxima*, yet exhibit intraspecific variation in hollowness. These solid types from Buffels Bay were collected in deeper water (8-16m) compared to other South African locations (ca. 2m), which may present a suite of environmental factors influencing both hollowness and blade morphology.

However, the separation of the solid and hollow types in deep water Buffels is primarily based on blade morphology, rather than overall size (Fig. 4, Fig. 5), of which the latter is similar between the two. Additionally blade morphology does not appear to vary greatly in hollow (*E. maxima*) sporophytes (Fig 5, Fig 6). Erting et al. (2004) identified a lack of distinction between *Laminaria hyperborea* (Gunnerus) Foslie and *L. digitata* (Hudson) Lamouroux in Denmark and the Faroe Islands, based on stipe morphology in areas of overlapping distribution. The same study reconciled *Laminaria faeroensis* (Børgesen) Børgesen with *L. saccharina* (Linnaeus) J.V. Lamouroux, now *Saccharina latisimma* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W Saunders (Erting et al. 2004, Lane et al. 2006), highlighting the extent of intraspecific phenotypic variability in kelp, as well as the ambiguity of plastic characters in species delimitation of morphologically overlapping species.

Based on traditional species descriptions these subtidal plants from Buffels Bay cannot be definitively differentiated into either *E. maxima* or *E. radiata*, although both traits (stipe hollowness and overall size) show better success at other locations included in the study. Thus, hollow types are tentatively referred to as *E. maxima* and solid types as *E. radiata*, although this must be viewed with some caution, particularly at sites where the two species co-occur, and especially with regards to the unusual deep water Buffels plants.

Co-occurrence of *E. maxima* and *E. radiata*

The isolated population of what may be *E. radiata* in deep water at Buffels Bay (Stegenga et al. 1997) have been described as displaying morphology intermediate between *E. maxima* and *E. radiata* (Rothman et al. in press). However, molecular analyses could not confirm the presence of this species in Buffels Bay, where a specimen clustered within *E. maxima* with one analysis (ITS/rbcL) and within *E. radiata* (atp8/trnWI) in another (Rothman et al. in press). Bolton (2012) identified the presence of *E. maxima* populations alongside *E. radiata* lining gullies at the De Hoop Nature Reserve, where plants also appear to intergrade morphologically (pers obs.). Morphological delimitation of sporophytes clearly resembling *E. maxima* and *E. radiata* were supported by molecular analyses indicating genetic distinction of the two species (Rothman et al. in press), although molecular analyses on plants of indeterminate morphology have not been undertaken.

De Hoop

De Hoop sporophytes appear to represent a more general morphology for both species (Fig. 6), from which both hollow and solid plants branch off, each distinguished from those at De Hoop by a number of different morphological features (Table 4). *Ecklonia radiata* from Dwesa and solid types from De Hoop display variable morphology (Fig. 8, Fig. 9f-h), although visually some overlap between the two locations may also be seen. *Ecklonia maxima* from shallow Buffels and hollow types from De Hoop display overlap in both size and frond morphology (Fig 9a-b, Fig. 10).

On observation the plants at De Hoop intergrade morphologically (Fig. 6, Fig. 9). The *E. radiata* specimens found there are not typical of the spinose form (Bolton 2010), although a small number are

present, while all sporophytes are generally smaller than those in shallow water at Buffels. Hollow plants (*E. maxima)* display some variation in size (Fig. 9a-b), despite occurring at similar depth gradients, visually causing confusion between small *E. maxima* and *E. radiata*. The smooth bladed solid type (*E. radiata)* at De Hoop contributes to the lack of distinction.

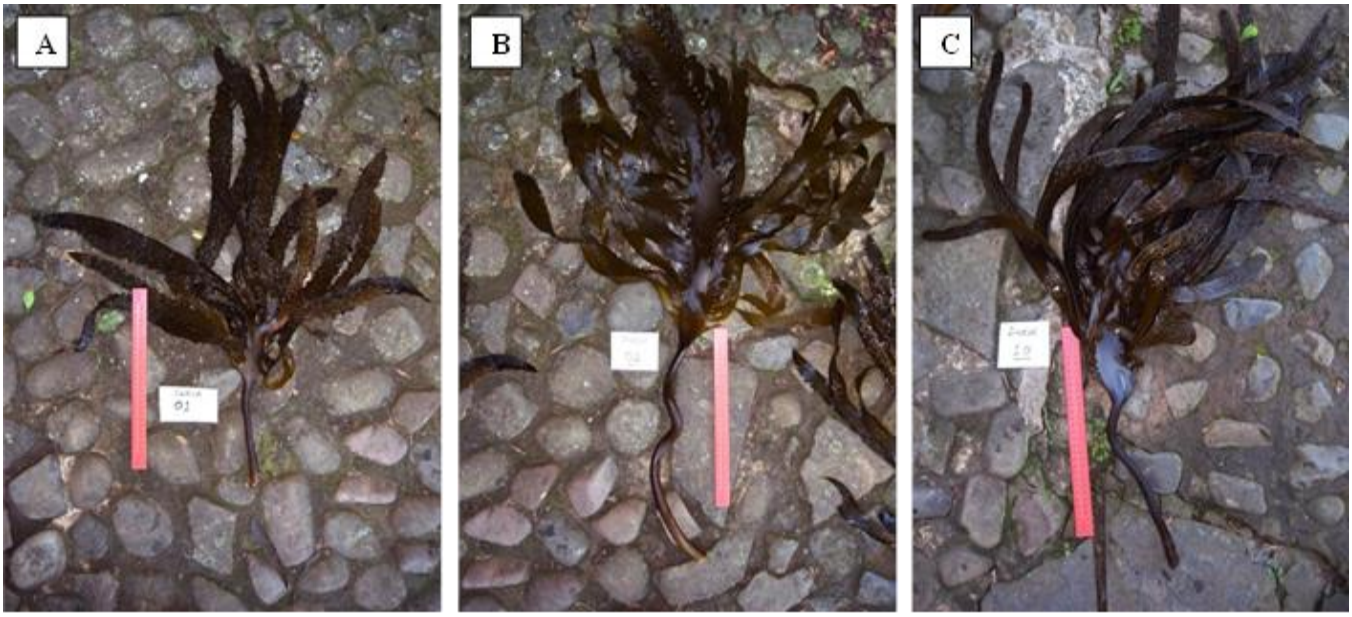

Figure 8: Plants representative of the range of morphologies (A-C) in *Ecklonia radiata* at Dwesa. Scales in all figures (red ruler) are 30cm.

Morphometrically however, the solid form does separate from the hollow. The spread of individual plants along a distinct size gradient indicates two clusters, one of generally smaller, solid-stiped plants and another of larger hollow plants (Fig. 6). This, in combination with the overlap between known *E. maxima* and *E. radiata* specimens, provides support for the presence of fairly distinct *E. maxima* and *E. radiata* populations within this location, if stipe hollowness is an appropriate trait to differentiate between species within *Ecklonia*. Given that the spinose form of *E. radiata* is not typical at De Hoop, and collection of all plants at the same depth gradient and under similar environmental conditions with regards to wave exposure, an important trait influencing frond morphology (Roberson et al. 2004, Wernberg and Thomsen 2005, Wernberg et al. 2010), the lack of distinction on a blade morphology basis is not entirely surprising.

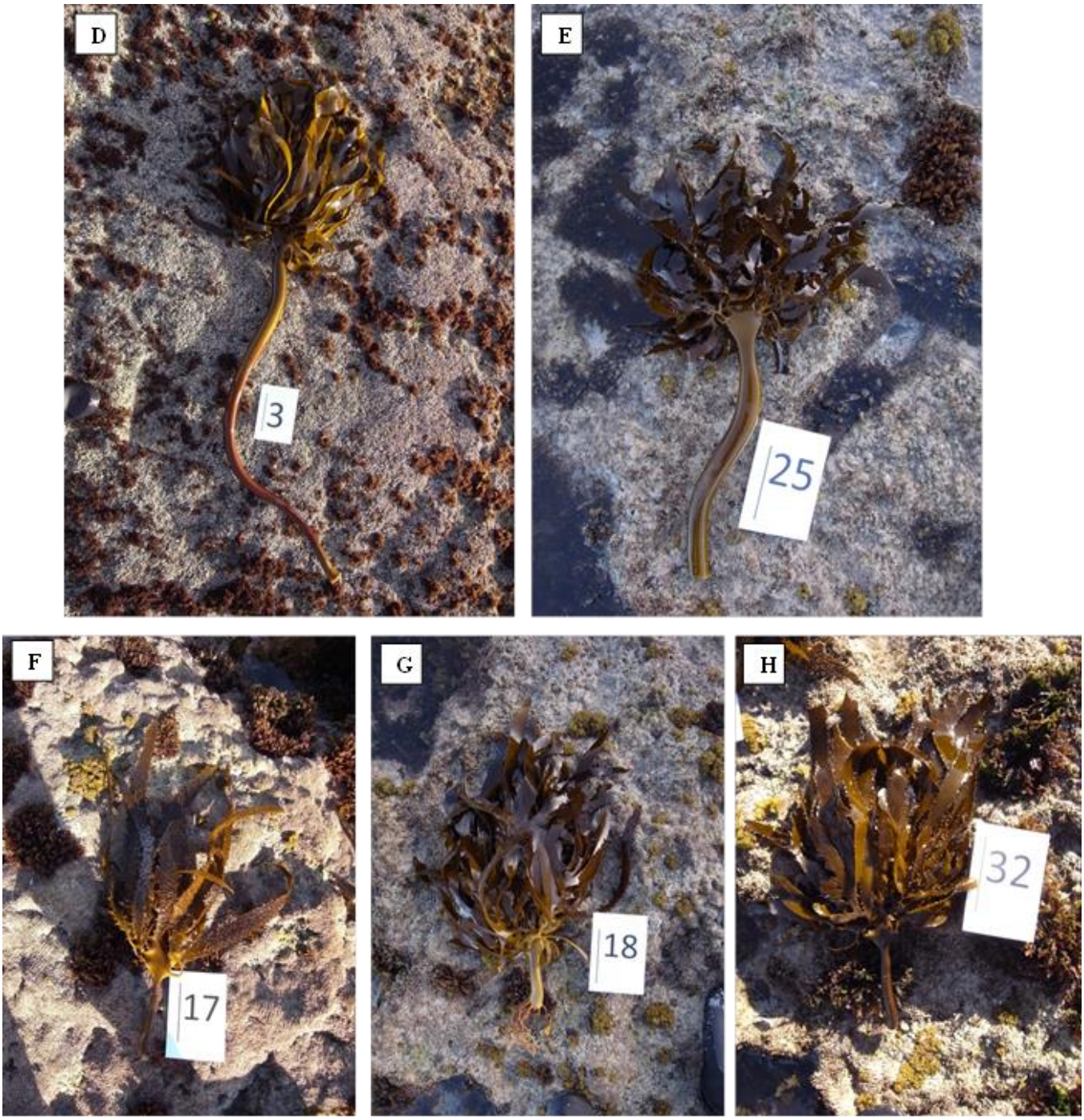

Figure 9: Plants representative of the range of morphologies in *Ecklonia* at the De Hoop Nature Reserve. Possible *E. maxima* plants (hollow stipe) are shown in (D and E), and possible *E. radiata* (solid stipe) in (F, G, H). Scales in all figures are 10cm

There is the possibility for hybrids to exist in nature (Bolton and Anderson 1987), however based on morphometrics it does not appear that the two species, or at least hollow and solid types, overlap to the extent that might be expected in hybrid morphology. This is especially true with regards to overall size, one of the factors confusing identification of the two species in the field. Given that *E. radiata*

appears to be highly variable in response to environmental factors (Fowler-Walker et al. 2006), and that *E. maxima* possibly evolved from an *E. radiata* type (Bolton and Anderson 1994, Matson et al. 2006), intraspecific variation of both species brought about by the local environment is likely present at De Hoop.

However, both types occur under similar environmental conditions, where the maintenance of a morphometric distinction between populations of hollow and solid types, provides some evidence for the separation of these two species morphologically at De Hoop. However, without genetic analyses the presence of hybrids cannot be ruled out, especially in light of the variability in the relationship between environmental and genetic control of morphology in kelp (Roberson and Coyer 2004, Miller et al. 2000, Fowler-Walker et al. 2006)

Buffels Bay

The species status of deep water Buffels plants is arguably the most confusing of all locations examined in this study, on the basis of both morphology and genetics. A great deal of morphological difference between shallow and deep water plants (Table 4) was seen, despite some obvious size variation within shallow water specimens (Fig. 4, Fig. 6 Fig. 10). Morphological overlap between hollow and solid plants is most evident here, appearing to be of a similar overall size (Fig. 6), but distinguishable by differences in blade morphology (Fig 4, Fig. 6). In particular, rugosity and secondary blade width separate deep water Buffels plants from all locations (Fig.5, Table 4). Rothman et al. (in press) found similar patterns in deep water blade morphology, where plants had wide, rugose fronds and a general *E. maxima*-like appearance.

Variation in blade morphology is often an adaptation to local water motion (Wernberg and Vanderklift 2010, Roberson and Coyer 2004, Wernberg and Thomsen 2005), where many species of macroalage have flat, strap-like blades in turbulent waters (Koehl et al. 2008) with lower associated drag (Denny 1988), and wide, rugose blades in low flow areas (Koehl et al. 2008), increasing turbulence around the blade, decreasing boundary layer thickness and increasing nutrient uptake (Wheeler 1980, Roberson and Coyer 2004). Concurrently, *E. arborea* in low flow environments were

found to have significantly more bullate blades than those in high flow environments, a genetically fixed trait (Roberson and Coyer 2004), while transplant experiments of giant bull kelp (*Nereocystis leutkeana*) with both flat, strap-like and wide, ruffled morphologies between flow environments exhibit a morphologically plastic response to exposure (Koehl et al. 2008).

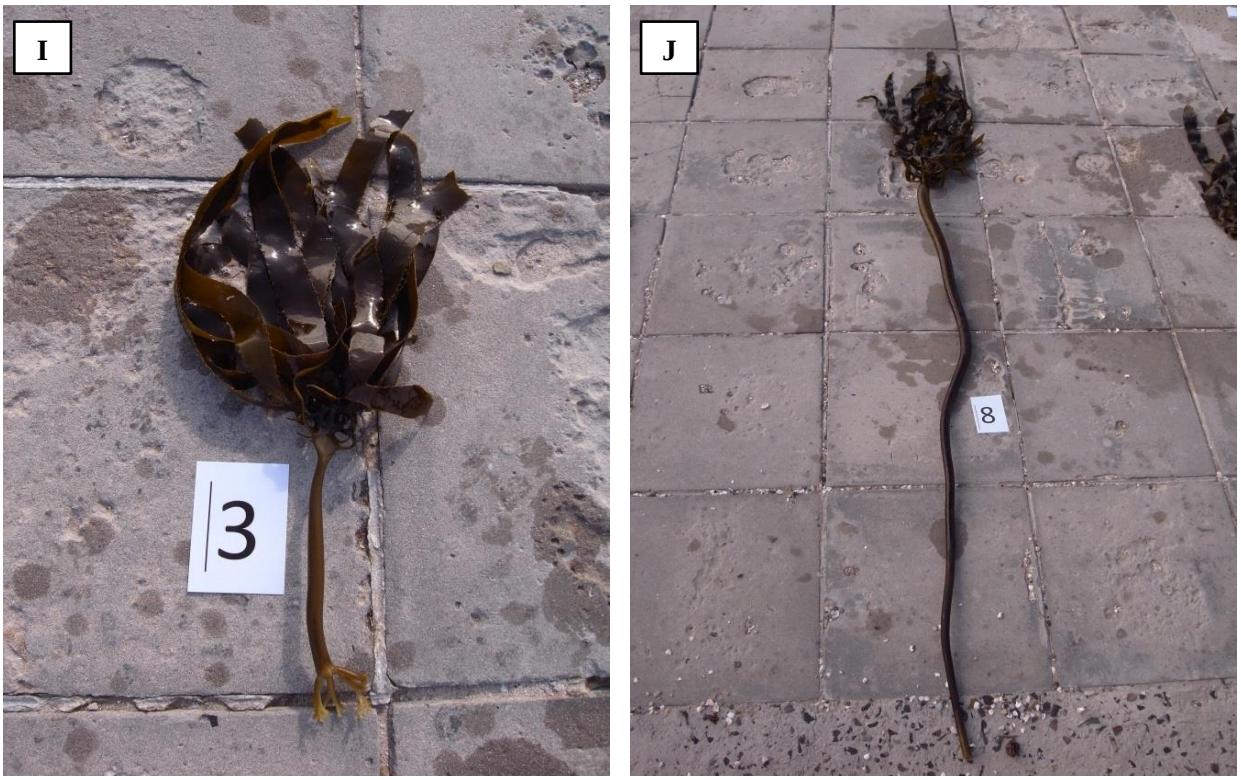

Figure 10: Plants representative of the range of morphologies (I and J) in *Ecklonia maxima* in shallow water at Buffels Bay. Scales in figure I is 10cm.

Higher wave exposure and more turbulent flow in shallow environments in comparison to deeper habitats may explain the presence of the highly rugose, wide blades found in solid, deep water plants, causing the *E. radiata* like appearance of the blades. Interestingly, Australian *E. radiata* also grows subtidally (Wernberg and Vanderklift 2010), yet morphological differences between these specimens and deep water Buffels plants is high (Fig.6, Table 4), re-iterating the strange nature of these plants. Plants from De Hoop and Dwesa also exhibit rugose blades at shallow depths, in areas of high wave exposure, which may indicate that the rugose blades of deep water Buffels plants arise from a genetic association with *E. radiata* rather than depth. Some rugosity in Australian *E. radiata* confuses the

distinction between depth and species factors, highlighting the importance and need for environmental and genetic data to confirm the cause of rugosity in these specimens.

The lack of clarity in genetic studies (Rothman et al. in press), and the overlap in morphology between hollow and solid types in deep water Buffels specimens as well as the evident difference between shallow (known *E. maxima*) and deep water plants, may be seen as indicators for the occurrence of morphologically plastic *E. radiata* in Buffels Bay. Differences in morphology from Australian and South African *E. radiata* as well as solid types from De Hoop (Fig. 6) could be explained by both the absence of *E. radiata* in deep water at Buffels Bay, as well as the presence of hybrids between the two species.

Morphologically we can neither confirm nor refute the presence of *E. radiata* in Buffels Bay, although we have identified a morphological difference between shallow and deep water plants, and between known *E. radiata* and solid specimens. Without further genetic analyses, especially those aimed at hybrid identification, the nature of these strange subtidal plants remains unclear. Furthermore, the confounding effect of environment, especially depth, on these plants will need to be addressed, as kelp morphology varies in response to abiotic controls through both genetically fixed and plastic responses.

Variability in *E. radiata*

Significant differences among locations confirm that *E. radiata* shows morphological variation among populations both within South Africa, and between continents (Fig. 6). However, sporophytes typically show more overlap in morphology with each other than with hollow types. Overall size characters (Fig. 4) are lower and less variable for known *E. radiata* specimens, and solid types in general are smaller in size than hollow types (Fig. 6). This supports descriptions of the two species, where *E. radiata* is typically a much smaller kelp than *E. maxima* (Bolton and Anderson 1987, Stegenga et al. 1997).

Despite variable blade morphology in solid plants (Fig. 6, Fig. 8, Fig. 9, Fig. 10, Fig. 11) these traits show success in distinguishing populations of *E. radiata* (Table 4, Fig. 5) and solid types from hollow

and *E. maxima* plants. These differences arise primarily from the presence of longer primary blades with a greater number of secondaries and spines, typical *E. radiata* traits (Wing et al. 2007, Wernberg et al. 2010), while rugosity, primary blade twists and secondary blade width cause the divergence of solid Buffels plants (Fig.6). South African solid types are distinguished from hollow more prominently on a size basis, rather than frond morphology (Table 4, Fig. 6).

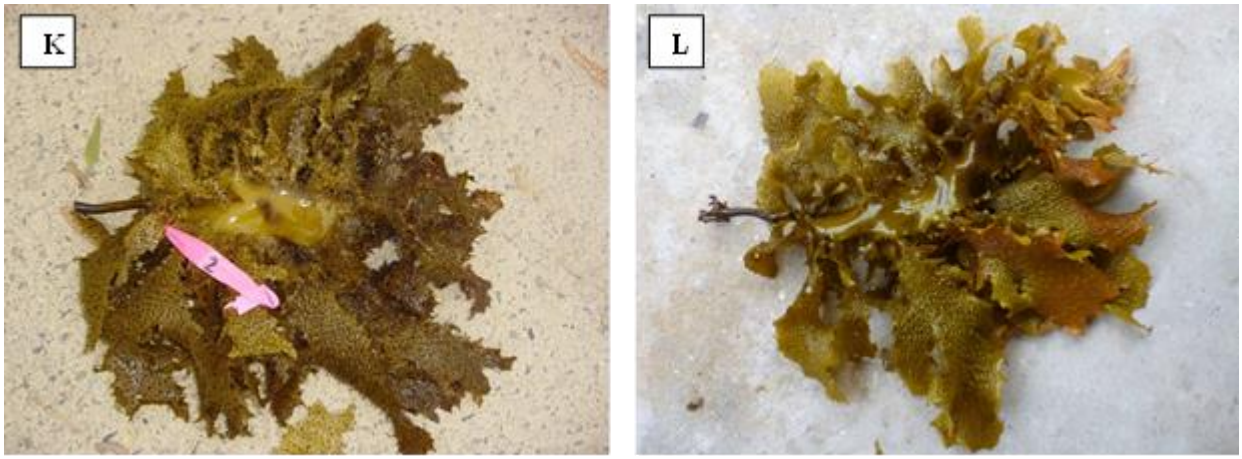

Figure 11: *Ecklonia radiata* plants from Australia. Specimen (K) is from New South Wales, Australia. Specimen (L) is from Rottnest Island, off Fremantle near Perth in Western Australia. Both resemble kelps found at Mewstone Rock, WA.

Spinuosity is known to vary in *E. radiata* (Bolton and Anderson 1994, Wing et al. 2007, Wernberg and Vanderklift 2010, Martin and Zuccarello 2012, Bolton et al. 2012), evidenced in this study (Table 4, Fig. 8,Fig. 9f-h, Fig. 11). Despite variation in both rugosity and spinuosity between Australian sporophytes, they still appear morphologically more similar to each other compared to the South African *E. radiata* and solid types (Table 4, Fig.6). This can be seen across size and frond morphology (Fig. 4, Fig. 5, Fig. 6), although visually (Fig. 8a, Fig. 9f, Fig. 11) some overlap in morphology between continents is observed.

We can therefore confirm the presence of morphological variation within *E. radiata* in both South Africa and Australia. A distinction in overall morphology between continents is observed, prominent on both blade morphology and size, while within South Africa all solid types cluster based on size.

Potential *E. radiata* from deep water at Buffels Bay is distinguished primarily on the basis of blade morphology.

Conclusions

Significant morphological differences as well as visual patterns in PCA and nMDS analyses (Fig.6) provide good evidence for the separation of hollow and solid types of *Ecklonia*, attributable to the morphological differentiation of *E. radiata* and *E. maxima*. These differences confirm traditional species descriptions for the most part, although overall size rather than stipe length appears to be a better predictor of species. Morphological variation of hollow plants (*E. maxima*) occurs primarily along a size gradient, while solid plants (*E. radiata*) show greater variation in blade morphology, although distinctions between populations on a size basis are also seen. Variation within *E. radiata* is evident, and despite morphometric clustering of solid plants, a distinction between Australian and South African *E. radiata* is observed. Overall size distinctions between hollow and solid types at De Hoop provide support for the morphological separation of these two species at this location, although visually confusing in the field. The population of subtidal solid plants from Buffels Bay clusters most strongly with South African solid plants on the basis of size, but their blade morphology is very different from all others examined in this study. Without further genetic analyses on these specimens their species status remains unclear, as either *E. maxima*, *E. radiata* or as hybrids between them.

Morphological variation in macroalgae may be under genetic or environmental control, or a combination of the two. Thus, for more complete resolution of the nature of morphological variation within and between these two species more detailed genetic analyses, especially those aimed at hybrid identification are needed. The importance of using more than one molecular marker to elucidate phylogenetic relationships within kelps has been identified (Lane et al. 2006, McDevit and Saunders 2010) therefore future *Ecklonia* studies may consider a combination of DNA sequencing across nuclear, chloroplastic and mitochondrial markers (Rothman et al. in press) as well as DNA barcoding methods (McDevit and Saunders 2010) to investigate genetic differences among and within populations of both species, while multilocus DNA fingerprinting (Roberson and Coyer 2004) has

shown some success in hybrid identification. Additionally, data on environmental parameters known to influence morphology will help clarify these effects on individual morphological characters.

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APPENDIX

Table 5: Eigenvector values of variables making up the first two PC's (Fig. 6)

Variable	PC1	PC2
Thallus Length	0.382	-0.07
Thallus Weight	0.361	-0.076
Stipe Length	0.372	-0.159
Stipe Diameter	0.332	0.107
PB Length	-0.233	-0.233
PB Width	0.217	-0.002
PB Thickness	0.35	-0.207
PB Twists	-0.082	0.283
No. SB	-0.223	-0.223
SB Length	0.365	0.156
SB Width	0.054	0.594
SB Thickness	0.108	-0.119
Rugosity	-0.071	0.554
Spinousity	-0.204	-0.162

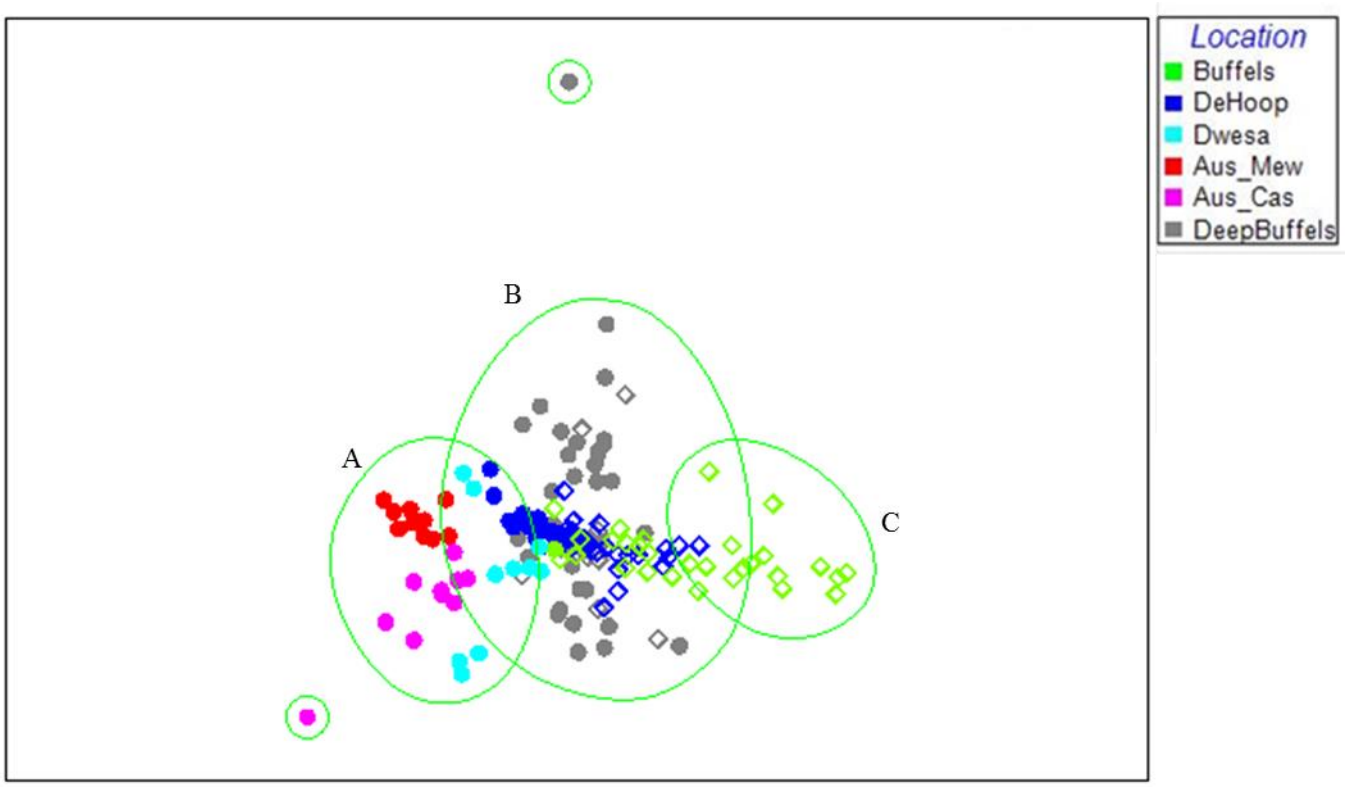

Figure 12: nMDS analysis of 14 morphological variables identified in Table 2 for all sampling locations. Key indicates sampling locations (See Table 3). Stress= 0.14. Each point represents a single

plant. The presence (◇) or absence (●) of a hollow stipe is indicated. Green lines indicate groups (A, B, C) identified from resemblance levels (distance = 5.6).

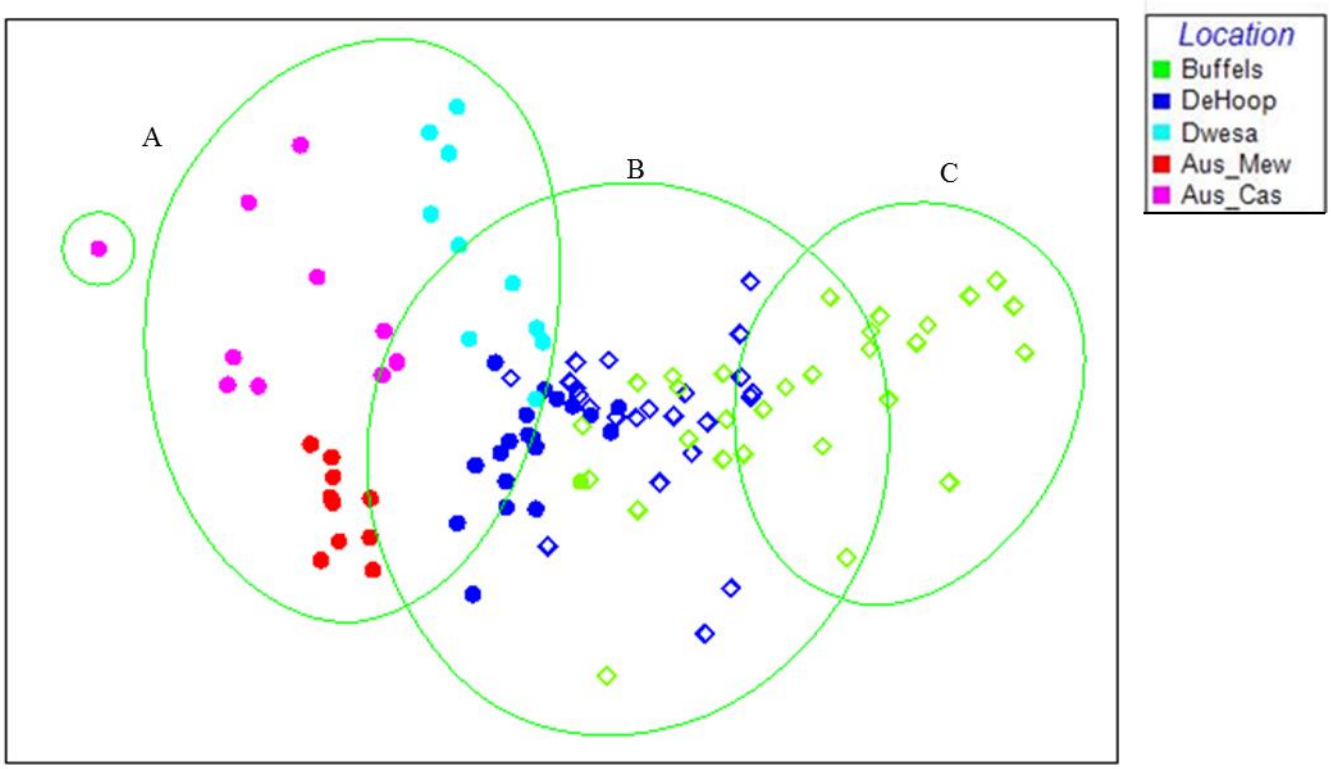

Figure 13: nMDS analysis of 14 morphological variables identified in Table 2 for sampling locations indicated in Table 3, excluding deep water Buffels Bay plants. Key indicates sampling locations (See Table 3). Stress= 0.12. Each point represents a single plant. The presence (◇) or absence (●) of a hollow stipe is indicated. Green lines indicate groups (A, B, C) identified from resemblance levels (distance = 5.6).